Increased susceptibility of serum amyloid A 1.1 to degradation by MMP-1: potential explanation for higher risk of type AA amyloidosis

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Objective. Genetic polymorphisms in serum amyloid A (SAA) have been shown to substantially influence the risk of developing type AA amyloidosis. Recently, a role for MMP-1 has been suggested in the pathogenesis of AA amyloidosis. Therefore, we investigated if the SAA1 isotypes are differentially degraded by MMP-1.

Methods. Degradation of different SAA isotypes by MMP-1 was assessed by immunoblotting. MALDI-TOF mass spectrometry was used to identify degradation fragments.

Results. We found that SAA1.5 is more resistant to degradation by MMP-1 than SAA1.1. This difference is caused by the capacity of MMP-1 to cleave at the site of the polymorphism at position 57.

Conclusion. These results may explain the higher risk of amyloidosis in patients with a SAA1.1/1.1 genotype vs SAA1.5/1.5 or SAA1.1/1.5 genotype. In addition, the impaired degradation of SAA1.5 by MMP-1 could also explain the higher serum SAA concentrations in persons with a SAA1.5 genotype.

KEY WORDS: AA amyloidosis, Matrix metalloproteinase.

Introduction

Type AA amyloidosis is a feared complication of chronic inflammatory conditions. It is caused by the deposition of insoluble fibrillar amyloid proteins in the extracellular spaces in a variety of organs and tissues [1, 2]. The AA protein that forms the amyloid fibril in type AA amyloidosis is mainly derived from degradation products of serum amyloid A type 1 (SAA1).

SAA1 is an 12 kDa protein of 104 amino acids that is produced mainly in the liver after stimulation by various pro-inflammatory cytokines, and is considered to be part of the acute phase response [3]. The amyloid fibrils found in patients with AA amyloidosis largely consist of the N-terminal 76 amino acids of SAA, although N-terminal fragments of different lengths have been reported [4–8].

Although a prolonged elevation of SAA1 is a prerequisite for the development of amyloidosis, only a small portion of patients with chronic inflammation will ever develop type AA amyloidosis [9]. Polymorphisms in the gene coding for SAA1 have been identified as a factor that influences the risk of developing amyloidosis. Two single nucleotide polymorphisms at exon 3 constitute three different isotypes: SAA1 1.1 (Val52–Ala57), SAA1 1.3 (Ala52–Ala57) and 1.5 (Ala52–Val57). Caucasian patients with a 1.1/1.1 genotype have a higher risk of developing amyloidosis compared with patients with a 1.5 genotype, but the reason for this is unknown. Recently, a role of MMPs was suggested in the pathogenesis of amyloidosis. MMPs are enzymes that modulate the extracellular matrix and are present in AA amyloid deposits [10]; SAA1 induces production of MMPs by mononuclear phagocytes and synovial fibroblasts [11, 12]. Furthermore, MMP-1, -2 and -3 have been shown to degrade both SAA and AA fibrils in vitro [13]. Interestingly, these MMP degrade SAA1 preferentially in the region spanning amino acids 52–58.

In this study, we investigated to what extend the degradation of SAA1 by MMP1 is dependent on the SAA1 isotype.

Materials and methods

Preparation of SAA1.1 and SAA1.5

A recombinant human SAA 1.1 expression system using pET21a plasmid and BL21 Escherichia coli has already been established [14]. SAA 1.5 cDNA was prepared from SAA1.1 by the PCR mutagenesis method [15]. SAA was purified by molecular sieve chromatography followed by chromatofocusing or hydrophobic interaction chromatography [14]. Purity and identity were examined after purification, by amino-terminal sequencing.

Degradation of SAA by recombinant MMP-1

Degradation experiments were performed with minor modification as described by Stix et al. [13]. Briefly, 5 µl of concentrated SAA (5 µg/µl, dissolved in 4 M of urea) was mixed with 25 µl of reaction buffer (20 mM Tris, 150 mM NaCl, 5 mM CaCl2 and 1 mM ZnCl2, pH 7.6) and 5 µl of purified human MMP-1 (Sigma-Aldrich, St Louis, MO, USA) at a final concentration of 0.35 µM. The reaction was performed at 37°C and stopped after 2, 24 and 48 h by addition of 5 µl of 25 mM EDTA. Incubation without MMP-1 or in the presence of 3.57 mM EDTA served as negative control. The degradation experiments were performed as three independent experiments.

SDS–PAGE and western blotting

After degradation the samples were subjected to discontinuous Tris–Tricine SDS–PAGE [16]. Proteins were visualized by staining with Coomassie Brilliant blue. For western blotting, proteins were transferred from unstained gels onto a nitrocellulose membrane (pore size: 0.22 µm) and incubated with anti-SAA antibodies (clone 86.1 and clone 86.5, dilution 1:500) for 4 h at room temperature. Monoclonal antibodies against SAA1 were a kind gift of Johan Bijzet, University Medical Center Groningen, Groningen, the Netherlands. The membrane was rinsed and incubated with rat anti-mouse IgG antibodies conjugated with horseradish peroxidase. Immunostaining was visualized with 3,3'-diaminobenzidine tetrahydrochloride. The stained gels were

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scanned with a densitometer (Umax, Uden, the Netherlands). Images from the densitometer were analysed using Totallab software (Nonlinear Dynamics, Newcastle, UK).

**Mass spectrometry**

Degradation fragments of SAA exposed to MMP-1 were characterized by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Biflex III, Bruker, FRG) using the linear mode of operation. SAA was degraded as described above and 1 μl aliquots were removed at 2, 24 and 48 h. Aliquots were mixed with 5 μl of trifluoroacetic acid (TFA, 0.1% v/v) and 5 μl of matrix solution (10 mg of sinapic acid in 1 ml of a 50:50 mixture of acetonitrile) and 0.1% TFA, (v/v). A volume of 1 μl from this mixture was spotted on the target plate. Spectra were acquired in the linear mode as described [17]. The BioTools 2.0 software (Bruker Daltonics, Germany) was used for the annotation of the SAA fragments; the accepted mass tolerance was 100 p.p.m.

**Statistical analysis**

Data were analysed by the unpaired Student’s t-test using SPSS 14.0 for Windows.

**Results**

The first experiment was performed in order to assess the difference in the rate of degradation of SAA1.1 and SAA1.5 by MMP-1. Purified human recombinant SAA1.1 and SAA1.5 were incubated with 0.35 μM of MMP-1 for 24 h and 48 h and subsequently subjected to SDS–PAGE. Quantitative analysis showed that both the isotypes were susceptible to degradation, but SAA1.1 was degraded to a greater extent (Fig. 1). At 48 h MMP-1 degraded SAA1.1 to 48.9% of the total amount added, while full-length SAA1.5 was only reduced to 70.8% (P < 0.05). In addition, there was a different pattern of degradation. SAA1.1 co-incubation with MMP-1, results in appearance of two degradation products of ~5 kDa and ~6.5 kDa. These fragments do not appear after treatment of SAA1.5 (Fig. 1b). Immunoblot analysis showed that the two bands reacted with anti-SAA antibody.

In addition, immunoblot analysis, which has a higher sensitivity than SDS–PAGE, shows three faint bands in the degradation of SAA1.5 at ~9 kDa, 8 kDa and 5 kDa (Fig. 1c).

Next, we used MALDI-TOF MS to further investigate the degradation profile of SAA1.1 and SAA1.5 (Fig. 2). We were able to identify the two degradation products of ~6.5 kDa as SAA1.1 fragment 1–57 and ~5 kDa as 58–104 indicating MMP 1 cleaves SAA1.1 between residue Ala57–Ile58. These fragments most likely correspond to the 6.5 kDa and 5 kDa bands identified by Coomassie staining and immunoblotting.

In the degradation of SAA1.5, we could identify three fragments: 58–104, 30–104 and 24–104. These fragments most likely correspond with the three faint bands of 5 kDa (58–104), 8 kDa (30–104) and 9 kDa (24–104) identified by immunoblot analysis (Fig. 1c).

**Discussion**

This study shows that SAA1.5 is largely resistant to degradation by MMP-1, contrary to SAA1.1. This difference is determined by the capacity of MMP-1 to cleave at residues 57 and 58 of the protein, which are either Val57–Ile58 (SAA1.5) or Ala57–Ile58 (SAA1.1). This results in the emergence of different degradation products (Figs 1 and 2).

The difference in capacity of MMP-1 to degrade the two isoforms of SAA1 could explain the differential risk of developing amyloidosis. Based on sequencing of AA amyloid fibril proteins, SAA1.1 was proposed as a risk factor for AA amyloidosis [18]. Numerous studies confirmed that patients with an SAA1.1/1.1 genotype have a strongly increased risk of developing amyloidosis not only in patients with RA, but also in patients with familial Mediterranean fever, juvenile chronic arthritis and Behcêt’s disease [19–26]. Caucasian patients with a 1.1/1.1 genotype have a 3- to 7-fold increased risk of amyloidosis compared with other genotypes [20, 22, 26, 27]. The SAA1.3 isotype, found to have a 4.5-fold increased risk of amyloidosis in the Japanese population [10, 29], has an alanine at position 57 similar to SAA1.1; the increased risk of amyloidosis in patients with SAA1.3 could be caused by the same mechanism as in SAA1.1.

Our data suggest a putative role of MMP-1 in the pathogenesis of amyloidosis. This is further supported by the observation that MMPs are found in close proximity of amyloid fibres, specifically intracellularly in cells surrounding amyloid deposits [10]. Furthermore, MMP-1 has been shown to degrade SAA in vitro [13].

We failed to detect the 30–104 and 24–104 degradation fragments of SAA 1.1 by MALDI-TOF (Fig. 2). We hypothesize that this is caused by further degradation of these fragments as SAA1.1 is readily degraded at Ala57–Ile58, while the Val57–Ile58 of SAA1.5 is largely resistant to degradation. Furthermore, MALDI-TOF MS is only a semi-quantitative analysis measuring relative abundance of various fragments in a sample. The much higher concentrations of 1–57 and 58–104 in the degradation of SAA1.1 could further limit the detection of small amounts of the 30–104 and 24–104 fragments. Figure 3 shows a schematic representation of SAA1.1 and SAA1.5 degradation.

Since amyloid fibrils are composed of N-terminal part of SAA, it is tempting to speculate that the 1–57 fragments that are produced in large quantities by MMP1 from SAA1.1 are directly incorporated into amyloid fibrils. Fragments of different lengths have been found in amyloid fibrils. Next to the AA protein that is composed of the first 76 amino acids of SAA, fragments of 5–12 kDa have been found [4–8]. However, to our knowledge, fragments that end at position 57 as part of AA amyloid deposits have not been described in the literature.

An alternative explanation for the increased risk of SAA1.1 genotype could be that the 1–57 fragments act as a source of amyloid-enhancing factor (AEF). Amyloidosis is considered a two-step process [1]. First, a critical mass of monomers that have
a β-sheet conformation has to be formed that can act as a nucleus (lag phase). Once the nucleus is formed there is rapid extension of fibrils. In mice, the lag phase, which takes 2–3 weeks, can be dramatically shortened to 24–48 h by administration of AEF [28–31]. AEF are small molecules with β-sheet propensity that can act as a nucleus for the generation and growth of AA amyloid fibrils similar to the action and propagation of prions [32–35]. Liu et al. [36] showed that synthetically produced N-terminal fragments of SAA have strong AEF activity, which was also shown by others [37]. Fragment 1–57 can be expected to conform to a β-sheet configuration [38], and thus act as AEF. Therefore, fragments resulting from MMP-1-mediated degradation of SAA1.1 could be more amyloidogenic than fragments from SAA1.5.

Interestingly, Migita et al. [39] found circulating fragments derived from SAA at much higher concentrations in RA patients with amyloidosis compared with RA patients without amyloidosis. While both groups had similar serum SAA concentrations, in the amyloidosis patients there was a considerable amount of SAA fragments that have a molecular weight of ~6 kDa, similar to the 1–57 fragment we found. These fragments may be produced by SAA degradation by MMPs.

In addition, our results may explain the earlier observations that RA patients with an SAA 1.5 genotype have higher serum SAA concentrations relative to CRP than patients with SAA 1.1 genotype [40]. Also, in healthy subject basal serum, SAA concentrations are significantly higher in persons who carry a SAA 1.5 allele, and is the highest in persons homozygote for SAA 1.5 [41]. Furthermore, when injected in mice, SAA1.1 and SAA1.3 are more rapidly cleared from the circulation than SAA1.5 [15]. These observations combined with our data might fit with a role for MMP-1 in the clearance of SAA from the circulation.

In conclusion, SAA1.1 is more susceptible to in vitro degradation by MMP-1 than SAA1.5, resulting in higher production of the 1–57 fragments from SAA1.1. This may explain the higher risk of AA amyloidosis in patients with a SAA1.1 genotype, and the higher serum SAA concentration in persons with an SAA1.5 genotype.

![MALDI-TOF MS analysis of the degradation products from SAA1.1 and SAA1.5.](image)

![Schematic representation of SAA1.1 and SAA1.5 degradation by MMP-1.](image)
Disclosure statement: The authors have declared no conflicts of interest.

References


